

1 LRH: T. FLATT AND T. J. KAWECKI

2 RRH: JUVENILE HORMONE AND COST OF REPRODUCTION

3

4 JUVENILE HORMONE AS A REGULATOR OF THE TRADE-OFF BETWEEN

5 REPRODUCTION AND LIFESPAN IN *DROSOPHILA MELANOGASTER*

6

7 THOMAS FLATT^{1,2,3} AND TADEUSZ J. KAWECKI^{1,4}

8

9 ¹*Department of Biology, Section of Ecology and Evolution,*

10 *University of Fribourg, Fribourg, Switzerland*

11

12 ²*Present Address: Department of Ecology and Evolutionary Biology,*

13 *Brown University, Providence, RI 02912, USA*

14

15 ³*thomas_flatt@brown.edu*

16

17 ⁴*tadeusz.kawecki@unifr.ch*

18

19 Correspondence to:

20 Thomas Flatt

21 Division of Biology and Medicine

22 Department of Ecology and Evolutionary Biology

23 Brown University

24 Box G-W

25 Providence, RI 02912, USA

26 Phone: (401) 863 6378

27 Fax: (401) 863 2166

28

29

- Re-revised Manuscript, March 2007 -

1 *Abstract.*—Trade-offs between reproduction and lifespan are ubiquitous, but little is
2 known about their underlying mechanisms. Here we combine treatment with the juvenile
3 hormone analog (JHa) methoprene and experimental evolution in *Drosophila*
4 *melanogaster* to study the potential role of juvenile hormone (JH) in mediating such
5 trade-offs at both the physiological and evolutionary level. Exposure to JHa in the larval
6 medium (and up to 24 hours posteclosion) increased early-life fecundity but reduced
7 lifespan of normal (unselected) flies, supporting the physiological role of JH in mediating
8 the trade-off. This effect was much smaller for lifespan, and not detectable for fecundity,
9 in fly lines previously bred for 19 generations on a medium containing JHa. Furthermore,
10 these selection lines lived longer than unselected controls even in the absence of JHa
11 treatment, without a detectable reduction in early-life fecundity. Thus, selection for
12 resistance to JHa apparently induced some evolutionary changes in JH metabolism or
13 signaling, which led to longer lifespan as a correlated response. This supports the
14 hypothesis that JH may mediate evolution of longer lifespan, but - contrary to our
15 expectation - this apparently does not need to trade off with fecundity.

16

17 *Keywords.*—*Drosophila*, cost of reproduction, experimental evolution, juvenile hormone,
18 reproduction, lifespan, trade-off, aging, antagonistic pleiotropy.

19

20

21

1 In many organisms reproduction trades off with survival (Williams 1966; Roff
2 1992). Such trade-offs may be observed at the physiological level (individuals that
3 reproduce more live shorter), and at the evolutionary (genetic) level (evolution of higher
4 reproductive effort is associated with reduced lifespan as a correlated response; Reznick
5 1985; Bell and Koufopanou 1986; Reznick 1992; Roff 1992; Stearns 1992). In the fruit
6 fly (*Drosophila melanogaster*), reproductive factors that physiologically shorten lifespan
7 include egg production (Partridge et al. 1987; Sgro and Partridge 1999), exposure to
8 males (Partridge and Fowler 1990), and mating (Fowler and Partridge 1989; Chapman et
9 al. 1995). Evolutionary trade-offs between fecundity and lifespan have been observed in
10 numerous selection experiments (Rose 1984; Zwaan et al. 1995; Partridge et al. 1999;
11 Stearns and Partridge 2001).

12 Little is known about the proximate mechanisms underlying these trade-offs (Leroi
13 2001; Barnes and Partridge 2003; Harshman and Zera 2007). A widely held view is that
14 they are mediated through competitive resource allocation (Reznick 1985; Bell and
15 Koufopanou 1986; van Noordwijk and de Jong 1986; Kirkwood and Rose 1991; de Jong
16 and van Noordwijk 1992). Under this view, reproduction shortens lifespan because it
17 withdraws limited resources that could otherwise be used for somatic maintenance and
18 repair. However, a direct causal role for resource allocation has not been conclusively
19 demonstrated (Rose and Bradley 1998; Barnes and Partridge 2003), and the observation
20 that survival and reproduction can be experimentally decoupled in the nematode worm
21 (*Caenorhabditis elegans*) and *D. melanogaster* is at odds with this notion (Hsin and
22 Kenyon 1999; Arantes-Oliveira et al. 2002; Tu and Tatar 2003; Partridge et al. 2005).

1 Alternatively, reproduction might accelerate damage-inducing processes independently of
2 resource allocation, but this possibility has rarely been tested (Tatar and Carey 1995;
3 Silberman and Tatar 2001; Barnes and Partridge 2003). Thus, specific genetic and
4 physiological mechanisms mediating reproduction-survival trade-offs have so far rarely
5 been identified (Rose and Bradley 1998; Leroi 2001; Barnes and Partridge 2003;
6 Partridge et al. 2005; Harshman and Zera 2007). Furthermore, it is not clear how often
7 trade-offs observed at the physiological and evolutionary level involve the same
8 proximate mechanisms (Stearns 1989, 1992).

9 Given their central role in regulating physiology (Mangelsdorf et al. 1995; Schwartz et
10 al. 2000), hormones are likely to be involved in modulating life history trade-offs
11 (Ketterson and Nolan 1992; Finch and Rose 1995; Dingle and Winchell 1997; Zera and
12 Harshman 2001; Flatt and Kawecki 2004; Flatt et al. 2005; Harshman and Zera 2007). In
13 insects, juvenile hormone (JH) is a major developmental and reproductive hormone,
14 which affects multiple physiological processes by regulating gene expression in a variety
15 of tissues (Nijhout 1994). Several lines of evidence suggest that JH stimulates
16 reproduction at the expense of shorter lifespan (Flatt et al. 2005; Tu et al. 2006). In
17 grasshoppers and butterflies, surgical removal of the corpora allata (glands producing JH)
18 induces reproductive diapause and dramatically extends lifespan (Pener 1972; Herman
19 and Tatar 2001; Tatar and Yin 2001). In *Drosophila*, mutants of the *Insulin-like Receptor*
20 (*InR*) gene or the insulin-like receptor substrate *chico* are JH-deficient, exhibit ovarian
21 arrest with nonvitellogenic oocytes, and are long-lived (Clancy et al. 2001; Tatar et al.
22 2001a; Tu et al. 2005). Similarly, in wild-type fruit flies undergoing reproductive

1 diapause, JH synthesis is down-regulated, ovarian development arrested, and
2 demographic senescence is reduced (Tatar and Yin 2001; Tatar et al. 2001b). When long-
3 lived *InR* mutants or diapausing flies are treated with the JH analog (JHa) methoprene,
4 survival is reduced and egg development restored (Tatar et al. 2001a,b). However, since
5 JH biosynthesis is also reduced in a sterile homozygous *InR* mutant genotype with
6 normal longevity, JH deficiency might not be sufficient to extend lifespan (Tatar et al.
7 2001a). Furthermore, JHa treatment of sterile JH-deficient *chico* mutants cannot restore
8 fecundity (Richard et al. 2005).

9 Despite much progress (Flatt et al. 2005; Tu et al. 2006), testing life history effects of
10 JH in small insects such as *Drosophila* remains challenging: (1) surgical removal of the
11 corpora allata is difficult; (2) JHa and JH synthesis inhibitors can have pharmacological
12 side effects (Wilson et al. 1983; Zera 2006); (3) null mutants of most genes involved in
13 JH biosynthesis have not yet been isolated (Belles et al. 2005); (4) the molecular
14 components of JH signal transduction remain largely unknown (Flatt and Kawecki 2004;
15 Wilson 2004; Flatt et al. 2005; Tu et al. 2006); and (5) measuring JH biosynthesis and
16 titers is difficult (Zera 2006). Consequently, how JH affects the trade-off between
17 reproduction and lifespan is still poorly understood (Harshman and Zera 2007). In
18 particular, it remains unclear whether JH modulates the trade-off between reproduction
19 and survival in reproductively active, non-JH-deficient wild-type flies. This is the first
20 issue we address in this paper.

21 Furthermore, the fact that hormonal manipulation (treatment with JH or JHa) and JH-
22 deficient mutants tend to have antagonistic physiological effects on reproduction and

1 survival does not automatically imply that changes in JH signaling or metabolism
2 mediate an evolutionary trade-off between these fitness components (Flatt et al. 2005;
3 Zera 2006). For that, two conditions must be satisfied. First, there must be additive
4 genetic variation affecting aspects of JH metabolism or signaling (Flatt and Kawecki
5 2004; Zera 2006). Second, this variation must have antagonistic effects on reproduction
6 and survival, in parallel to those caused by hormonal or genetic manipulations. It remains
7 unknown whether these conditions are satisfied (cf. Flatt 2004a); this is the second issue
8 we address in this paper.

9 Here we combined hormonal manipulation with experimental evolution to investigate
10 a potential role of JH in the evolutionary trade-off between reproduction and survival in
11 *D. melanogaster*. Aiming to induce evolutionary changes in JH metabolism or signaling,
12 we exposed experimental populations to selection for resistance to deleterious effects of
13 the JH analog (JHa) methoprene in the larval food medium. We hypothesized that such
14 evolved changes in JH metabolism or signaling would have two effects on adult survival
15 and fecundity. First, we expected the selected lines to become less sensitive than
16 unselected control lines to the effects of JHa on lifespan and fecundity. Second, we
17 predicted that selection for JHa resistance would lead to lower sensitivity of the flies to
18 their own JH. If so, and if JH signaling indeed mediates the reproduction-survival trade-
19 off, then the selected lines should show lower fecundity and longer lifespan than the
20 control lines even without JHa treatment.

21
22
23
24

MATERIAL AND METHODS

Methoprene as JH analog

1 JH or its synthetic analog (JHa) methoprene can disrupt development and increase
2 preadult mortality when applied throughout development or at a time when the hormone
3 is not normally present (Wilson and Fabian 1986; Riddiford and Ashburner 1991). For
4 that reason methoprene is used in insecticides; it is also widely used in insect physiology
5 because it mimics JH action, but is better soluble, more potent, and more resistant to *in*
6 *vivo* degradation than JH (Riddiford and Ashburner 1991; Wilson 2004; Zera and Zhao
7 2004). In particular, methoprene can act as a faithful JH agonist in a manner that is
8 qualitatively identical to that of JH, both *in vivo* and in cell culture (Cherbas et al. 1989;
9 Riddiford and Ashburner 1991; Wilson 2004; T. Flatt, unpublished data). We thus used
10 methoprene as an agent of selection.

11 While JHa can be applied topically to adults, application via the food medium
12 provides an easy and effective way of exposure. This method can specifically mimic JH
13 activity and is efficient in treating a large number of flies (Riddiford and Ashburner
14 1991). Individuals exposed to dietary JHa continuously receive JHa through the gut by
15 feeding, the cuticle by contact, and - since JHa produces a volatile vapor - the tracheal
16 system by respiration (Wilson and Chaykin 1985; Wilson and Fabian 1986; Riddiford
17 and Ashburner 1991; Wilson et al. 2003; T. G. Wilson, pers. comm.). Importantly,
18 exposure of larvae to JHa in the food medium allowed us to impose selection on JH
19 signaling or metabolism without directly selecting on adult survival, fecundity, or their
20 responses to JHa.

1 The effects of dietary JHa may depend on culture density, and it is thus possible that
2 feeding larvae degrade JHa, possibly by an enzyme in the saliva or the presence of gut
3 bacteria (Wilson and Chaykin 1985). To avoid confounding effects of density on the
4 effectiveness of JHa we therefore rigorously controlled larval densities in the selection
5 experiment and all assays (see below). However, in a pilot experiment with the base
6 stock, we found no evidence that the effects of JHa depend on larval density. When
7 testing viability as a function of both JHa concentration (control: no JHa; treatment: 2.08
8 μl JHa per ml) and egg density (100, 150, 200 eggs per bottle) we found that JHa induced
9 about 25% egg-to-adult mortality (two-way ANOVA; $F_{1,26} = 1084.7$, $P < 0.0001$)
10 irrespective of egg density ($F_{2,26} = 19.2$, $P = 0.79$). Furthermore, since JHa in the food
11 medium is also taken up by contact/vapor and has been found to mimic the action of JH
12 in several previous experiments (e.g., Wilson and Fabian 1986; Riddiford and Ashburner
13 1991; Wilson et al. 2003), it is unlikely that degradation can render dietary JHa fully
14 ineffective.

15 Another potential caveat is that JHa (or its metabolites) in the larval diet might
16 inhibit nutrient uptake or assimilation; differential effects of dietary JHa on adult life
17 history in control versus selected flies could thus be due to differences in nutrient uptake
18 or conversion efficiency rather than JH action per se. For example, malnutrition
19 (starvation) decreases survival and fecundity, while dietary restriction increases survival,
20 but lowers fecundity in *Drosophila* (Good and Tatar 2001; Tatar 2007). Thus, under both
21 conditions, fecundity is reduced; however, our results were inconsistent with these
22 alternatives (see below).

1 *Selection Lines*

2 We established eight JHa-resistant selection lines and eight JHa-susceptible control
3 lines of *D. melanogaster*, all originating from an outbred base stock initiated with about
4 1000 flies in July 2000 and maintained in a population cage with a generation time of 2.5
5 weeks. The JHa-resistant lines were bred on a medium containing JHa. JHa (methoprene;
6 Sigma-Aldrich; 1 µg/µl in 95% ethanol) was added to the still liquid, warm food medium
7 to a final concentration of 1.04 µl per ml medium. This dosage was chosen based on a
8 pilot dose-response experiment; it lowered egg-to-adult viability of the base stock by
9 about 13% (Flatt 2004b, Ph.D. Dissertation). JHa-susceptible control lines were
10 maintained under identical conditions, but were not exposed to JHa in the food medium.

11 For each of the 16 lines we established three culture bottles, with a controlled density
12 of 200 eggs per bottle. In each generation, 15 to 16 days after egg laying, we randomly
13 selected 30 females and 30 males from each bottle within a line. Adults from each bottle
14 within a line were pooled for mating and females were allowed to oviposit overnight. The
15 next day, we collected 600 eggs per replicate line and allocated them to a new set of three
16 culture bottles, 200 eggs per bottle, to initiate the next generation. The 15/16 days
17 generation time provided sufficient time for larval development and eclosion, allowing
18 almost all viable adults to eclose (control: 99.8%; selection: 99.0%; T. Flatt, unpublished
19 data). The base stock and all experimental lines were maintained at 25°C, on a 12h:12h
20 light:dark cycle, in bottles containing 25 ml of standard cornmeal-sugar-agar-yeast
21 medium.

22
23

1 *General Assay Methods*

2 We measured egg-to-adult viability, developmental time, body weight at eclosion,
3 early fecundity, adult survival, and age-specific mortality of all JHa-resistant and JHa-
4 susceptible lines under two test conditions: when raised on normal food medium and
5 when raised on the medium containing JHa. We used the same JHa concentration as that
6 used to impose selection (1.04 $\mu\text{l/ml}$); this is important since, if the assay environment
7 differs from the selection environment, results obtained from the assay may not
8 correspond to the situation in the population under selection (Ackermann et al. 2001).
9 Before carrying out life history assays, all lines were kept for two generations without
10 selection on normal food medium at controlled larval density to minimize parental
11 effects. To obtain the individuals to be assayed, 200-300 adult flies from each line were
12 placed into egg laying chambers containing plates with oviposition medium (a mixture of
13 agar and orange juice) and females were allowed to oviposit overnight. The next day, we
14 initiated 10 vials for each line, each vial with 50 eggs on 10 ml of normal food.

15
16 *Egg-to-Adult Viability*

17 To test for a direct response to selection, after 7, 14, and 19 generations we measured
18 egg-to-adult viability (proportion surviving) of all lines on normal medium and on
19 medium containing JHa. To set up a viability assay, we placed 200-300 adult flies per
20 replicate line into egg laying chambers overnight. The next day, eggs from each line were
21 allocated to 10 vials with normal food, and to 10 vials with food containing JHa (1.04
22 $\mu\text{l/ml}$), each vial with 50 eggs on 10 ml food ($n = 2$ selection regimes $\times 2$ JHa conditions
23 $\times 8$ replicate lines $\times 10$ vials = 320 vials). Vials were checked every 12 hours for eclosing

1 adults until all flies had emerged. We used repeated-measures multivariate analysis of
2 variance (MANOVA) implemented in JMP IN 5.1. (SAS Institute, Sall et al. 2004) to
3 determine the experiment-wide significance of main and interaction effects while
4 controlling for within-treatment covariance (von Ende 2001). Thus, since viabilities
5 within a given treatment might be correlated over time, among-treatment effects
6 (selection regime, JHa treatment, JHa \times regime) and within-treatment effects (time) are
7 coordinately evaluated using exact F values based on Roy's greatest root (Harris 1985).
8 Since sex ratio at eclosion was not affected by selection regime, JHa treatment, replicate
9 line nested within regime, or interactions between these factors (analysis not shown),
10 sexes were pooled for analysis of viability data.

11
12

Lifespan and Mortality

13 Adult survival and age-specific mortality were measured after 19 generations of
14 selection. To set up the lifespan assay, we collected newly eclosed adult flies within a 24
15 hour period. For each replicate selection and control line and each test condition, we
16 established one 1-liter population cage ($n = 2$ selection regimes \times 8 cages/lines \times 2 JHa
17 conditions = 32 cages). This factorial design allowed us to test for effects of selection
18 regime, JHa treatment, and the JHa \times regime interaction; however, we could not
19 separately estimate the effects of replicate cage versus replicate line.

20 Each cage was initiated with 50 newly eclosed adults, mixed sex (see Tatar et al.
21 2001a,b for cage design). Dead flies were removed from cages and scored every two
22 days, at which time fresh food was provided in a vial with 5 ml of standard cornmeal-
23 sugar-agar-yeast medium. Cages were maintained at 25°C, on a 12h:12h light:dark cycle.

1 Note that, irrespective of the larval medium and selection regime, flies were not exposed
2 to JHa during adulthood (except for up to 24 hours between eclosion and being collected
3 for flies raised on JHa-containing medium).

4 Survival data were pooled across replicate cages within a treatment. From these data
5 we constructed life tables by the extinct cohort method (Chaing 1984). Adult survival
6 (fraction of flies alive, l_x) was calculated as N_x/N_0 , where N_x is the number of flies alive at
7 the beginning of each census interval and N_0 is the initial cohort size. Data were analyzed
8 using Kaplan-Meier survival analysis implemented in JMP IN 5.1. (Sall et al. 2004);
9 significant differences in survival between pairs of cohorts were tested by using the log-
10 rank test (Parmar and Machin 1995).

11 To obtain additional insights into the pattern of mortality change, we estimated age-
12 specific instantaneous mortality rate as $\ln(\mu_x) \approx \ln(-\ln[1-D_x/N_x])$, where D_x is the number
13 of dead flies in a given census interval (Elandt-Johnson and Johnson 1980). Since in
14 many species, including *Drosophila*, mortality rates increase exponentially with age
15 (Carey et al. 1992; Curtsinger et al. 1992), we fitted a standard model describing such a
16 mortality trajectory to our data, namely the Gompertz model: $\mu_x = \lambda e^{\gamma x}$, where x is age,
17 λ is baseline mortality or “frailty”, and γ is the rate at which mortality increases as a
18 function of age x (Elandt-Johnson and Johnson 1980). The intercept parameter λ (frailty)
19 represents the individual susceptibility or “proneness” to death due to systems that
20 degenerate progressively with age; the slope parameter γ is interpreted as the rate of
21 aging, reflecting the progressive degeneration of somatic function within individuals. We
22 fitted Gompertz parameters to each cohort using maximum likelihood estimation (MLE)

1 implemented in WinModest (Pletcher 1999) and tested for differences in parameter
2 values among pairs of cohorts using log-likelihood ratio tests. To test for effects of
3 selection regime, JHa treatment, and JHa \times regime on mortality we used proportional
4 hazards analysis (Cox regression; Parmar and Machin 1995) implemented in JMP IN 5.1.
5 Analyzing survival and mortality patterns separately for females and males did not affect
6 the outcome of our analyses; similarly, proportional hazards analysis did not reveal a
7 significant sex \times JHa \times regime interaction (analyses not shown). We therefore pooled
8 survival and mortality data for both sexes.

9
10

Early Fecundity

11 For each population cage in the lifespan assay, we counted all eggs laid during the
12 first five 48 hour periods as estimates of early fecundity over the first 10 days after
13 eclosion ($5 \times 32 = 160$ vials). Age-specific daily fecundity was estimated as the average
14 number of eggs laid per female per 48 hour interval. When estimating fecundity, egg
15 counts were averaged over all reproductive females alive in a given 48 hour period. Data
16 on age-specific fecundity were analyzed using repeated-measures MANOVA
17 implemented in JMP IN 5.1. (Sall et al. 2004).

18
19

Developmental Time and Body Weight at Eclosion

20 Since effects of selection and/or JHa treatment on reproduction and lifespan might be
21 confounded by inadvertent effects on developmental time and body weight, we assayed
22 these traits after 14 generations of selection in all lines, both on normal food and on food
23 containing JHa. For both assays, 200-300 adult flies per line were placed into egg laying

1 chambers overnight. The next day, eggs from each line were allocated to 2 vials with
 2 normal food, and to 2 vials with food containing JHa (1.04 $\mu\text{l/ml}$), each vial with 50 eggs
 3 on 10 ml food ($n = 2$ selection regimes \times 2 JHa conditions \times 8 replicate lines \times 2 vials =
 4 64 vials). Vials were checked for eclosing adults twice a day from day 7 after egg laying.
 5 Average developmental time was calculated once all flies had eclosed. Within 12 hours
 6 of emergence, flies were frozen, dried for 3 days at 80°C, and weighed individually on a
 7 Mettler MT5 balance to an accuracy of 0.001 mg. Data for both traits were analyzed with
 8 JMP IN 5.1. (Sall et al. 2004), using a nested mixed-effects ANOVA model:

$$X = \mu + A_i + B_j + AB_{ij} + C(A)_{k(i)} + BC(A)_{jk(i)} + \text{error},$$

11

12 where μ = mean, A = selection regime (fixed factor, 2 levels: selection, control), B = JHa
 13 treatment (fixed factor, 2 levels: JHa, no JHa), C(A) = lines nested in selection regime
 14 (random factor, 8 levels: 8 independent replicate lines).

15

16

RESULTS

17

Egg-to-Adult Viability

18 JHa reduced egg-to-adult viability in unselected (JHa-susceptible) control flies, but
 19 not in selected (JHa-resistant) flies, suggesting that selected flies evolved significant
 20 levels of resistance to JHa (FIGURE 1, TABLE 1; JHa \times regime interaction, contrast
 21 between selected and control flies treated with JHa: exact $F_{1, 28} = 6.02$, $P = 0.02$). Egg-to-
 22 adult viability of resistant flies treated with JHa increased from 63% in generation 7 to
 23 71% in generation 19, whereas treatment of susceptible control flies with JHa decreased

1 their viability on average by 19% (average of 3 assays). Selected flies assayed on
2 medium without JHa did not have reduced egg-to-adult viability, indicating that JHa-
3 resistant flies did not pay a detectable viability cost of resistance (FIGURE 1; JHa × regime
4 interaction, contrast between selected and control flies without JHa: exact $F_{1,28} = 1.86$, P
5 = 0.18).

6
7

Lifespan and Mortality

8 Exposure to JHa during development strongly reduced subsequent adult survival and
9 life expectancy in control flies, but to a much lesser extent in JHa-resistant flies which
10 had greater survival than control flies (FIGURE 2A, TABLES 2 and 3). Thus, JHa reduced
11 the longevity of flies, but JHa-resistant flies evolved partial insensitivity to these lifespan
12 shortening effects.

13 JHa-resistant flies also evolved significantly extended lifespan relative to the JHa-
14 susceptible control flies in the absence of JHa (FIGURE 2A, TABLES 2 and 3). Thus,
15 evolutionary changes in JH metabolism or signaling due to selection for improved JHa
16 resistance caused lifespan extension in a normal environment. JHa treatment of long-
17 lived JHa-resistant flies restored median lifespan to the level seen in untreated control
18 flies (FIGURE 2A, TABLES 2 and 3; control flies without JHa: 44 days; long-lived JHa-
19 resistant flies: without JHa: 46 days, with JHa: 44 days).

20 Gompertz and proportional hazards analyses of age-specific mortality confirmed that
21 JHa shortens lifespan (FIGURE 2B, TABLE 2; Cox regression, effect of JHa: likelihood-
22 ratio $\chi^2 = 74.6$, $P < 0.0001$). JHa overall increased mortality early in life, but this effect
23 diminished with age, either because the flies cleared off JHa (it was not present in the

1 adult medium), or because the most susceptible individuals died first (FIGURE 2B). JHa
2 had different effects on mortality in unselected versus selected flies (Cox regression, JHa
3 \times regime: likelihood-ratio $\chi^2 = 19.4$, $P < 0.0001$). In JHa-susceptible control flies, JHa
4 treatment significantly increased frailty (λ), the baseline susceptibility to death, but
5 decreased the Gompertz slope parameter γ (FIGURE 2B, TABLE 2). In JHa-resistant flies,
6 JHa treatment did not affect mortality parameters, thus confirming that JHa-resistant flies
7 evolved insensitivity to the lifespan shortening effects of JHa (FIGURE 2B, TABLE 2).
8 Furthermore, JHa-resistant and JHa-susceptible flies were genetically differentiated with
9 respect to mortality parameters (FIGURE 2B, TABLE 2; Cox regression, selection regime:
10 likelihood-ratio $\chi^2 = 74.4$, $P < 0.0001$). In the absence of JHa, JHa-resistant flies showed
11 reduced frailty as compared to unselected control flies; in the presence of JHa, long-lived
12 JHa-resistant flies exhibited reduced frailty, but an increased rate of aging (FIGURE 2B,
13 TABLE 2).

14
15

Early Fecundity

16 JHa treatment significantly increased age-specific fecundity over the first 10 days of
17 adult life, thus confirming the well-known role of JH as a reproductive hormone (FIGURE
18 3, TABLE 4). However, selection regime and the JHa \times selection regime interaction did
19 not affect fecundity (TABLE 4). Contrasts analysis confirmed that control flies and
20 selected flies were not genetically differentiated in terms of early fecundity (FIGURE 3;
21 contrast, selected versus control flies, without JHa: exact $F_{1,28} = 0.11$, $P = 0.74$; with
22 JHa: exact $F_{1,28} = 0.16$, $P = 0.69$). While JHa significantly increased early fecundity of

1 JHa-susceptible control flies (FIGURE 3; contrast, exact $F_{1,28} = 5.08$, $P = 0.03$), fecundity
2 of JHa-resistant flies was insensitive to treatment with JHa (contrast, exact $F_{1,28} = 0.48$, P
3 $= 0.49$). These results also suggest that the lifespan shortening effects of JHa were likely
4 to be physiological since the same dosage of JHa positively affected fecundity in control
5 flies.

6 *Developmental Time and Body Weight at Eclosion*

8 JHa treatment increased developmental time of flies on average by 16.8 hours (6.7%)
9 as compared to flies assayed on normal food medium, yet selection regime and the JHa \times
10 regime interaction had no effect on this trait (FIGURE 4A, TABLE 5). JHa treatment
11 reduced body weight at eclosion by 13.5% (approximately 0.03 mg); however, the
12 selection regime and JHa \times regime interaction did not affect weight (FIGURE 4B, TABLE
13 6). Thus, the absence of correlated responses for both traits suggests that the prolonged
14 lifespan and the insensitivity to effects of JHa on reproduction and lifespan observed in
15 JHa-resistant flies is unlikely to be a consequence of selection on developmental time and
16 weight. Similarly, since JHa treatment had similar effects on developmental time and
17 weight at eclosion in JHa-susceptible control and JHa-resistant selected flies, flies in the
18 two selection regimes were unlikely to differ in nutrient uptake or assimilation.

19 DISCUSSION

21 Pleiotropic hormones are thought to be important regulators of life history trade-offs
22 (Tatar et al. 2003; Flatt and Kawecki 2004; Flatt et al. 2005; Harshman and Zera 2007).
23 In *Drosophila* and other insects, juvenile hormone (JH) has been proposed to stimulate

1 reproduction at the expense of survival (Flatt et al. 2005; Tu et al. 2006). This makes JH a
2 candidate target of natural selection on the trade-off between reproduction and survival; a
3 mechanism that could mediate evolutionary shifts of the life history. Several aspects of
4 our results provide support for this hypothesis.

5 First, our results confirm the physiological role of JH in stimulating reproduction and
6 reducing survival (Flatt et al. 2005; Tu et al. 2006). We extend previous results by
7 showing that this effect occurs in reproductively active, non-JH-deficient wild-type flies,
8 and even if the JH treatment is limited to the larval stage and the first 24 hours after adult
9 eclosion. Supplementing larval food medium with the JH analog (JHa) methoprene
10 increased fecundity of unselected JHa-sensitive flies, but reduced their lifespan,
11 confirming the antagonistic physiological effect of JH on reproduction and survival (Flatt
12 et al. 2005; Tu et al. 2006). Inspection of mortality rates suggested that the effect of JHa
13 treatment on mortality was particularly strong within the first 2-3 weeks of adult life, and
14 became progressively smaller at later ages. This is confirmed by the Gompertz model:
15 JHa treatment significantly increased the Gompertz intercept parameter λ (frailty) of JHa-
16 sensitive lines, but reduced their Gompertz slope parameter γ . However, this does not
17 necessarily mean that JHa treatment slowed down the rate of aging. The simplest
18 explanation for this pattern is that the effect of JHa simply wore off with age, as the flies
19 cleared it out of their system (JHa was only added to the larval medium and not re-
20 applied during adult life). This result is consistent with the observation that removal of
21 the corpora allata extends lifespan in butterflies mainly by reducing frailty, whereas JH
22 treatment of allatectomized individuals increases frailty (Herman and Tatar 2001).

1 Second, we found that flies can evolve reduced sensitivity to the effects of JHa.
2 Lines maintained on a JHa-containing medium evolved partial resistance to the adverse
3 effects of JHa on egg-to-adult viability. As we had hypothesized, these JHa-resistant flies
4 also became less sensitive to the physiological effects of JHa treatment on reproduction
5 and lifespan. The effect of JHa treatment on adult survival was much smaller in JHa-
6 resistant lines than in unselected JHa-sensitive lines. When developing on JHa-containing
7 medium, JHa-resistant flies lived substantially longer than JHa-susceptible flies, and only
8 slightly shorter than JHa-resistant flies bred without JHa. In contrast to JHa-sensitive
9 lines, larval JHa treatment did not detectably increase fecundity of JHa-resistant lines, in
10 line with our predictions. Thus, while we could not find statistical evidence for a
11 correlated fecundity response to selection, JHa treatment seemed to promote fecundity in
12 JHa-susceptible flies, but not in JHa-resistant flies. Our results demonstrate that the base
13 population from which our selection lines were derived harbored heritable variation for
14 the response to JHa. This variation not only allowed the selected lines to improve their
15 egg-to-adult viability on a JHa-containing medium, but also led to reduced sensitivity to
16 the effect of JHa on lifespan and reproduction. Importantly, these effects on reproduction
17 and lifespan were not confounded by physiological effects of JHa on developmental time
18 and weight at eclosion or by inadvertent selection on these traits: JHa treatment affected
19 both traits similarly in control and selected flies, and neither trait showed a correlated
20 response to selection.

21 Third, and most interestingly, as a correlated response JHa-resistant lines evolved
22 lower adult mortality in the absence of JHa treatment (reduced frailty parameter of the

1 Gompertz model). As a result, their lifespan in the absence of JHa was on average 3.8
2 days (9.6 %) longer than that of unselected JHa-susceptible lines. We hypothesized that
3 selection for JHa resistance would induce compensatory changes which would effectively
4 reduce JH metabolism or signaling, with effects on life history resembling those of mild
5 JH deficiency. The longer lifespan of the JHa-resistant flies is consistent with this
6 hypothesis: extension of lifespan is typically observed in JH-deficient flies (Tatar and Yin
7 2001; Tatar et al. 2001a,b). However, JH-deficient flies also typically show impaired
8 ovarian development or reduced fecundity (Tatar and Yin 2001; Tatar et al. 2001a,b; Flatt
9 et al. 2005), which we did not observe in our JHa-resistant lines. Thus, our results support
10 the notion that changes in JH metabolism or signaling may mediate evolutionary changes
11 in lifespan, but they do not provide evidence that these changes would also mediate the
12 evolutionary trade-off with fecundity.

13 We can only speculate why JHa-resistant lines were able to extend their lifespan
14 without a concomitant reduction in fecundity. Two general mechanisms might account
15 for the antagonistic physiological effects of JH on reproduction and survival (Tatar and
16 Carey 1995; Barnes and Partridge 2003). On the one hand, JH might direct the allocation
17 of energy (nutrients) towards reproduction, thereby withdrawing limited resources from
18 investment into somatic maintenance and repair. On the other hand, JH might promote
19 reproductive processes that directly accelerate damage-inducing processes independent of
20 resource allocation. Although we cannot presently distinguish between these alternatives,
21 recent evidence suggests that JH promotes reproduction, but is a negative regulator of

1 stress resistance and immune function (Salmon et al. 2001; Tatar et al. 2001b; Rolff and
2 Siva-Jothy 2002; Rantala et al. 2003; Flatt et al. 2005; Tu et al. 2006).

3 Fecundity is, however, not always negatively correlated with longevity. A
4 heterozygous mutant genotype of *chico* (*chico*¹/*chico*⁺) is JH-deficient and long-lived, but
5 has a normal number of ovarioles (Clancy et al. 2001; Tu et al. 2005). Similarly, adult
6 wild-type flies that were yeast-deprived as third instar larvae exhibit reduced JH synthesis
7 at eclosion, decreased ovariole number and fecundity, but show normal rates of aging (Tu
8 and Tatar 2003). Thus, there is growing evidence that reproduction and survival can be to
9 some degree uncoupled, and that the tradeoff between these two traits is highly context-
10 dependent (Barnes and Partridge 2003; Partridge et al. 2005). Since many trade-offs are
11 condition-dependent (e.g., Stearns 1989, 1992, and references therein), it is possible that,
12 under benign lab conditions (i.e., high nutrition), a slight increase in investment into
13 somatic maintenance and survival would not require diverting resources from
14 reproduction. Indeed, the long-lived *Drosophila* mutant *Indy* only exhibits reduced
15 fecundity on a reduced-calorie diet, but not under normal (high calorie) rearing
16 conditions (Marden et al. 2003). Similarly, certain mutants of *C. elegans age-1* and *daf-2*
17 are long-lived without paying a fitness cost under normal laboratory conditions, but
18 fitness costs of longevity become apparent when these mutants are exposed to nutritional
19 stress or competed against a wild-type strain (Walker et al. 2000; Jenkins et al. 2004).

20 The effects of dietary application of JHa seen in our experiment were likely due to its
21 JH activity. JH analogs can specifically mimic JH action in *Drosophila* (both in flies and
22 cell culture) and other insects (Cherbas et al. 1989; Riddiford and Ashburner 1991;

1 Wilson 2004). Importantly, effects of dietary JHa application typically recapitulate those
2 of topical application (Wilson and Fabian 1986; Riddiford and Ashburner 1991; Wilson
3 et al. 2003). In support of this, the natural compound JH III reduced the viability of our
4 unselected JHa-susceptible flies, but JHa-resistant selected flies were insensitive to this
5 effect (data not shown; Flatt 2004b, Ph.D. Dissertation). Furthermore, we found that the
6 JHa concentration used in our experiment and assays increased fecundity of control flies.
7 Thus, this dosage had a physiological effect consistent with the well-known role of JH in
8 regulating vitellogenesis, ovarian maturation, and fecundity (Nijhout 1994; Hoffmann
9 1995; Gäde et al. 1997; Flatt et al. 2005). While we cannot rule out that JHa selectively
10 killed individuals with low fecundity, it is more parsimonious to assume that JHa
11 treatment increased fecundity in control flies due its pro-reproductive, JH-like action.

12 Our results add to a growing number of studies showing that hormones are involved
13 in mediating life history trade-offs in a variety of organisms (Ketterson and Nolan 1992;
14 Finch and Rose 1995; Dingle and Winchell 1997; Zera and Harshman 2001; Flatt et al.
15 2005; and references therein). In insects, selection experiments suggest that JH regulates
16 the trade-off between flight capability and reproduction in crickets (Zera and Zhao 2004;
17 Zera 2006), and quantitative genetic experiments with *Drosophila methoprene-tolerant*
18 (*Met*) mutants link JH signaling with life history pleiotropy (Flatt and Kawecki 2004).
19 Moreover, application of JH or JHa in flies and beetles promotes reproductive processes
20 at the expense of stress resistance or immune function (Salmon et al. 2001; Rolff and
21 Siva-Jothy 2002; Rantala et al. 2003); it also mediates the trade-off between gonad
22 development and eye-span (a secondary sexual trait) in stalk-eyed flies (Fry 2006).

1 Our findings have broad implications, beyond JH signaling in *Drosophila* and other
2 insects. JH functions downstream of insulin/IGF-1 signaling, an evolutionarily conserved
3 nutrient sensing pathway coordinating growth, reproduction, diapause, and aging in
4 animals as diverse as *C. elegans*, *Drosophila*, and rodents (Tatar et al. 2003). While *C.*
5 *elegans* and rodents do not produce JH, similar hormones downstream of insulin/IGF-1
6 might regulate reproduction and longevity in these organisms. Recent work has identified
7 two lipophilic hormones that modulate the effects of the reproductive system on lifespan
8 in *C. elegans* (Motola et al. 2006; Broué et al. 2007); in rodents, thyroid hormone might
9 play a similar role (Tatar et al. 2003; Flatt et al. 2006). These findings strongly suggest
10 that the endocrine regulation of trade-offs, such as between reproduction and lifespan, is
11 evolutionarily conserved. However, trade-offs at the physiological level do not
12 necessarily imply the existence of evolutionary trade-offs. For example, while some
13 physiological trade-offs might be genetically variable and contribute to an evolutionary
14 trade-off, others might be fixed and lineage-specific (Stearns 1989, 1992). Interestingly,
15 while our results suggest that JH is a proximate mechanism underlying the trade-off
16 between reproduction and lifespan, we could not convincingly show that JH signaling
17 actually mediates the evolutionary trade-off between these traits. Nonetheless, our results
18 indicate that *Drosophila* populations harbor genetic variation that affects JH signaling or
19 metabolism (cf. Flatt 2004a; Flatt and Kawecki 2004), and that this genetic variation may
20 mediate the evolution of longer lifespan. The rapid progress made by molecular
21 biologists in identifying candidate mechanisms affecting life history traits enables

1 evolutionary biologists to determine whether there is standing genetic variance for such
2 mechanisms in natural populations and whether they are under selection.

3
4
5

ACKNOWLEDGEMENTS

6 We thank Marc Tatar and Thomas G. Wilson for support and advice; Frederic Mery,
7 Ludwika Sygnarski, and Nicole Vouilloz for assistance in the laboratory; and Daniel
8 Promislow, Mohamed Noor, and two anonymous referees for helpful comments on the
9 manuscript. This research was supported by grants from the Swiss National Science
10 Foundation and the Roche Research Foundation to TJK.

11
12
13

LITERATURE CITED

- 14 Ackermann, M., R. Bijlsma, A. C. James, L. Partridge, B. J. Zwaan, and S.C. Stearns.
15 2001. Effects of assay conditions in life history experiments with *Drosophila*
16 *melanogaster*. *J. Evol. Biol.* 14:199-209.
- 17 Arantes-Oliveira, N., J. Apfeld, A. Dillin, and C. Kenyon. 2002. Regulation of life-span
18 by germ-line stem cells in *Caenorhabditis elegans*. *Science* 295:502-505.
- 19 Barnes, A. I., and L. Partridge. 2003. Costing reproduction. *Anim. Behav.* 66:199-204.
- 20 Bell, G., and V. Koufopanou. 1986. The cost of reproduction. Pp. 83-131 *in* R. Dawkins
21 and M. Ridley, eds. *Oxford Surv. Evol. Biol.* Vol.3. Oxford Univ. Press, Oxford,
22 UK.
- 23 Belles, X., D. Martin, and M. D. Piulachs. 2005. The mevalonate pathway and the
24 synthesis of juvenile hormone in insects. *Ann. Rev. Entomol.* 50:181-199.

- 1 Broué, F., P. Liere, C. Kenyon, and E.-E. Baulieu. 2007. A steroid hormone that extends
2 the lifespan of *Caenorhabditis elegans*. *Aging Cell* 6:87–94.
- 3 Carey, J. R., P. Liedo, D. Orozco, and J. W. Vaupel. 1992. Slowing of mortality rates at
4 older ages in large medfly cohorts. *Science* 258:457–461.
- 5 Chaing, C. L. 1984. *The life table and its applications*. Krieger, Malabar, Florida.
- 6 Cherbas, L., M. M. Koehler, and P. Cherbas. 1989. Effects of juvenile hormone on the
7 ecdysone response of *Drosophila* Kc cells. *Dev. Genet.* 10:177-188.
- 8 Chapman, T., L. F. Liddle, J. M. Kalb, M. F. Wolfner, and L. Partridge. 1995. Cost of
9 mating in *Drosophila melanogaster* females is mediated by male accessory gland
10 products. *Nature* 373:241-244.
- 11 Clancy, D. J., D. Gems, L. G. Harshman, S. Oldham, H. Stocker, E. Hafen, S. J. Leivers,
12 and L. Partridge. 2001. Extension of life-span by loss of CHICO, a *Drosophila*
13 insulin receptor substrate protein. *Science* 292:104-106.
- 14 Curtsinger, J. W., H. H. Fukui, D. R. Townsend, and J. W. Vaupel. 1992. Demography of
15 genotypes: failure of the limited life-span paradigm in *Drosophila melanogaster*.
16 *Science* 258:461–463.
- 17 de Jong, G., and A. J. Van Noordwijk. 1992. Acquisition and allocation of resources -
18 genetic (co) variances, selection, and life histories. *Am. Nat.* 139:749–770.
- 19 Dingle, H., and R. Winchell, R. 1997. Juvenile hormones as a mediator of plasticity in
20 insect life histories. *Arch. Insect Biochem. Physiol.* 35:359-373.
- 21 Elandt-Johnson, R., and N. L. Johnson. 1980. *Survival models and data analysis*. Wiley,
22 New York.

- 1 Finch, C. E., and M. R. Rose. 1995. Hormones and the physiological architecture of life
2 history evolution. *Quart. Rev. Biol.* 70:1-52.
- 3 Flatt, T. 2004a. Assessing natural variation in genes affecting *Drosophila* lifespan. *Mech.*
4 *Ageing Dev.* 125:155-159.
- 5 Flatt, T. 2004b. Effects of juvenile hormone on trait architecture in *Drosophila*
6 *melanogaster*. Ph.D. Dissertation, Department of Biology, Unit of Ecology and
7 Evolution, University of Fribourg, Switzerland, January 2004. Imprimerie St. Paul
8 (Fribourg), 209 pp.
- 9 Flatt, T., and T. J. Kawecki. 2004. Pleiotropic effects of *methoprene-tolerant (Met)*, a
10 gene involved in juvenile hormone metabolism, on life history traits in *Drosophila*
11 *melanogaster*. *Genetica* 122:141-160.
- 12 Flatt, T., M.-P. Tu, and M. Tatar. 2005. Hormonal pleiotropy and the juvenile hormone
13 regulation of *Drosophila* development and life history. *BioEssays* 27:999-1010.
- 14 Flatt, T., L. L. Moroz, M. Tatar, and A. Heyland. 2006. Comparing thyroid and insect
15 hormone signaling. *Integr. Comp. Biol.* 46:777-794.
- 16 Fowler, K., and L. Partridge. 1989. A cost of mating in female fruitflies. *Nature* 338:760-
17 761.
- 18 Fry, C. L. 2006. Juvenile hormone mediates a trade-off between primary and secondary
19 sexual traits in stalk-eyed flies. *Evolution & Development* 8:191-201.
- 20 Gäde, G., K. H. Hoffmann, and J. H. Spring. 1997. Hormonal regulation in insects: facts,
21 gaps, and future directions. *Physiol. Rev.* 77:963-1032.

- 1 Good, T. P., and M. Tatar. 2001. Age-specific mortality and reproduction to adult dietary
2 restriction in *Drosophila melanogaster*. *J. Insect Physiol.* 47:1467-1473.
- 3 Harris, R. J. 1985. A primer of multivariate statistics. Academic Press, New York.
- 4 Harshman, L. G., and A. J. Zera. 2007. The cost of reproduction: the devil in the details.
5 *Trends Ecol. Evol.* 22:80-86.
- 6 Herman, W. S., and M. Tatar. 2001. Juvenile hormone regulation of aging in the
7 migratory monarch butterfly. *Proc. Roy. Soc. London B* 268:2509-2514.
- 8 Hoffmann, K. H. 1995. Oogenesis and the female reproductive tract. Pp. 1-32 in S. R.
9 Leather and J. Hardy, eds. *Insect Reproduction*. CRC Press, New York.
- 10 Hsin, H., and C. Kenyon. 1999. Signals from the reproductive system regulate the
11 lifespan of *C. elegans*. *Nature* 399:362-366.
- 12 Jenkins, N. L., G. McColl, and G. J. Lithgow. 2004. Fitness cost of extended lifespan in
13 *Caenorhabditis elegans*. *Proc. Roy. Soc. London B* 271:2523-2526.
- 14 Ketterson, E. D., and V. Nolan. 1992. Hormones and life histories: an integrative
15 approach. *Am. Nat.* 140:S33-S62.
- 16 Kirkwood, T. B., and M. R. Rose. 1991. Evolution of senescence: late survival sacrificed
17 for reproduction. *Phil. Trans. Roy. Soc. London B (Biol. Sci.)* 332:15-24.
- 18 Leroi, A. 2001. Molecular signals versus the Loi de Balancement. *Trends Ecol. Evol.*
19 16:24-29.
- 20 Mangelsdorf, D. J., C. Thummel, M. Beato, P. Herrlich, G. Schutz, K. Umesono, B.
21 Blumberg, P. Kastner, M. Mark, P. Chambon, and R. M. Evans. 1995. The nuclear
22 receptor superfamily: the second decade. *Cell* 83:835-839.

- 1 Marden, J. H., B. Rogina, K. L. Montooth, and S. L. Helfand. 2003. Conditional tradeoffs
2 between aging and organismal performance of *Indy* long-lived mutant flies. Proc.
3 Natl. Acad. Sci. USA 100:3369-3373.
- 4 Motola, D., C. L. Cummins, V. Rottiers, K. Sharma, K. Sunino, E. Xu, R. Auchus, A.
5 Antebi, and D. Mangelsdorf. 2006. Identification of hormonal ligands for DAF-12
6 that govern dauer formation and reproduction in *C. elegans*. Cell 124:1209-1223
- 7 Nijhout, H. F. 1994. Insect hormones. Princeton Univ. Press, Princeton, New Jersey.
- 8 Parmar, M. K. B., and D. Machin. 1995. Survival analysis: a practical approach. Wiley,
9 Chichester, UK.
- 10 Partridge, L., and K. Fowler. 1990. Nonmating costs of exposure to males in female
11 *Drosophila melanogaster*. J. Insect Physiol. 36:419-425.
- 12 Partridge, L., D. Gems, and D. J. Withers. 2005. Sex and death: what is the connection?
13 Cell 120:461-472.
- 14 Partridge, L., A. Green, and K. Fowler. 1987. Effects of egg-production and of exposure
15 to males on female survival in *Drosophila melanogaster*. J. Insect Physiol 33:745-
16 749.
- 17 Partridge, L., N. Prowse, and P. Pignatelli. 1999. Another set of responses and correlated
18 responses to selection on age at reproduction in *Drosophila melanogaster*. Proc.
19 Roy. Soc. London B 266:255-261.
- 20 Pener, M. P. 1972. The corpus allatum in adult acridids: the inter-relation of its functions
21 and possible correlations with the life cycle. Pp. 135-147 in C. F. Hemming and T.

- 1 H. C. Taylor, eds. Proc. Int. Study Conf. Curr. Fut. Prob. Acridol. Centre for
2 Overseas Pest Research, London, UK.
- 3 Rantala, M. J., A. Vainikka, and R. Kortet. 2003. The role of juvenile hormone in
4 immune function and pheromone production trade-offs: a test of the
5 immunocompetence handicap principle. Proc. Roy. Soc. London B 270:2257-2261.
- 6 Reznick, D. 1985. Costs of reproduction - an evaluation of the empirical evidence. Oikos
7 44:257-267.
- 8 Reznick, D. 1992. Measuring the costs of reproduction. Trends Ecol. Evol. 7:42-45.
- 9 Richard, D. S., R. Rybczynski, T. G. Wilson, Y. Wang, M. L. Wayne, Y. Zhou, L.
10 Partridge, and L. G. Harshman. 2005. Insulin signaling is necessary for
11 vitellogenesis in *Drosophila melanogaster* independent of the roles of juvenile
12 hormone and ecdysteroids: female sterility of the *chico*¹ insulin signaling mutation is
13 autonomous to the ovary. J. Insect Physiol. 51:455-464.
- 14 Riddiford, L. M., and M. Ashburner. 1991. Effects of juvenile hormone mimics on larval
15 development and metamorphosis of *Drosophila melanogaster*. Gen. Comp.
16 Endocrin. 82:172-183.
- 17 Rolff, J., and M. T. Siva-Jothy. 2002. Copulation corrupts immunity: a mechanism for a
18 cost of mating in insects. Proc. Nat. Acad. Sci. USA 99:9916-9918.
- 19 Rose, M. R. 1984. Laboratory evolution of postponed senescence in *Drosophila*
20 *melanogaster*. Evolution 38:1004-1010.
- 21 Rose, M. R., and T. J. Bradley. 1998. Evolutionary physiology of the cost of
22 reproduction. Oikos 83:443-451.

- 1 Sall, J., L. Creighton, and A. Lehman. 2004. JMP start statistics. Thomson Learning,
2 Duxbury Press.
- 3 Salmon, A. B., D. B. Marx, and L. G. Harshman. 2001. A cost of reproduction in
4 *Drosophila melanogaster*: stress susceptibility. *Evolution* 55:1600-1608.
- 5 Schwartz, M. W., S. C. Woods, D. Porte, R. J. Seeley, and D. G. Baskin. 2000. Central
6 nervous system control of food intake. *Nature* 404:661-671.
- 7 Sgro, C. M., and L. Partridge. 1999. A delayed wave of death from reproduction in
8 *Drosophila*. *Science* 286:2521-2524.
- 9 Silbermann, R., and M. Tatar. 2000. Reproductive costs of heat shock protein in
10 transgenic *Drosophila melanogaster*. *Evolution* 54:2038-2045.
- 11 Stearns, S. C. 1989. Trade-offs in life-history evolution. *Funct. Ecol.* 3:259-268.
- 12 Stearns, S. C. 1992. The evolution of life histories. Oxford Univ. Press, Oxford, UK.
- 13 Stearns, S.C., and L. Partridge. 2001. The genetics of aging in *Drosophila*. Pp. 353-368
14 in E. J. Masoro and S. N. Austad, eds. *Handbook of the Biology of Aging*, 5th
15 edition. Academic Press (Elsevier), San Diego.
- 16 Tatar, M. 2007. Diet restriction in *Drosophila melanogaster*: design and analysis.
17 *Interdiscip. Top. Gerontol.* 35: 115-136
- 18 Tatar, M., and J. R. Carey. 1995. Nutrition mediates reproductive trade-offs with age-
19 specific mortality in the beetle *Callosobruchus maculatus*. *Ecology* 76:2066-2073.
- 20 Tatar, M., and C.-M. Yin. 2001. Slow aging during insect reproductive diapause: why
21 butterflies, grasshoppers and flies are like worms. *Exp. Geront.* 36:723-738.

- 1 Tatar, M., A. Bartke, and A. Antebi. 2003. The endocrine regulation of aging by insulin-
2 like signals. *Science* 299:1346-1351.
- 3 Tatar, M., S. A. Chien, and N. K. Priest. 2001b. Negligible senescence during
4 reproductive dormancy in *Drosophila melanogaster*. *Am. Nat.* 158:248-258.
- 5 Tatar, M., A. Kopelman, D. Epstein, M.-P. Tu, C.-M. Yin, and R. S. Garofalo. 2001a. A
6 mutant *Drosophila* insulin receptor homolog that extends life-span and impairs
7 neuroendocrine function. *Science* 292:107-110.
- 8 Tu, M.-P., and M. Tatar. 2003. Juvenile diet restriction and the aging and reproduction of
9 adult *Drosophila melanogaster*. *Aging Cell* 2:327-333.
- 10 Tu, M.-P., T. Flatt, and M. Tatar. 2006. Juvenile and steroid hormones in *Drosophila*
11 *melanogaster* longevity. Pp. 415-448 in E. J. Masoro and S. N. Austad, eds.
12 Handbook of the Biology of Aging, 6th edition. Academic Press (Elsevier), San
13 Diego.
- 14 Tu, M.-P., C. M. Yin, and M. Tatar. 2005. Mutations in insulin signaling pathway alter
15 juvenile hormone synthesis in *Drosophila melanogaster*. *Gen. Comp. Endocrin.*
16 142:347-356.
- 17 van Noordwijk, A., and G. de Jong. 1986. Acquisition and allocation of resources: their
18 influence on variation in life history tactics. *Am. Nat.* 128:137-142.
- 19 von Ende, C. N. 2001. Repeated-measures analysis: growth and other time-dependent
20 measures. Pp. 134-157 in S.M. Scheiner and J. Gurevitch, eds. Design and analysis
21 of ecological experiments, 2nd edition. Oxford Univ. Press, Oxford, UK.

- 1 Walker, D. W., G. McColl, N. L. Jenkins, J. Harris, and G. J. Lithgow. 2000. Evolution
2 of lifespan in *C. elegans*. *Nature* 405:296-297.
- 3 Williams, G. C. 1966. Natural selection, the costs of reproduction, and a refinement of
4 Lack's principle. *Am. Nat.* 100:687-690.
- 5 Wilson, T. G. 2004. The molecular site of action of juvenile hormone and juvenile
6 hormone insecticides during metamorphosis: how these compounds kill insects. *J.*
7 *Insect Physiol.* 50:111-121.
- 8 Wilson, T. G., and D. Chaykin. 1985. Toxicity of methoprene to *Drosophila*
9 *melanogaster* (Diptera: Drosophilidae): a function of larval culture density. *J. Econ.*
10 *Entom.* 78:1208-1211.
- 11 Wilson, T. G., and J. Fabian. 1986. A *Drosophila melanogaster* mutant resistant to a
12 chemical analog of juvenile hormone. *Dev. Biol.* 118:190-201.
- 13 Wilson, T. G., S. DeMoor, and J. Lei. 2003. Juvenile hormone involvement in
14 *Drosophila melanogaster* male reproduction as suggested by the *Methoprene-*
15 *tolerant*²⁷ mutant phenotype. *Insect Biochem. Mol. Biol.* 33:1167-1175.
- 16 Wilson, T.G., M. H. Landers, and G. M. Happ. 1983. Precocene I and II inhibition of
17 vitellogenic oocyte development in *Drosophila melanogaster*. *J. Insect Physiol.* 29:
18 249-254.
- 19 Zera, A. J. 2006. Evolutionary genetics of juvenile hormone and ecdysteroid regulation in
20 *Gryllus*: a case study in the microevolution of endocrine regulation. *Comp. Biochem.*
21 *Physiol. A (Molec. Integr. Physiol.)* 144:365-379.

- 1 Zera, A. J., and L. G. Harshman. 2001. The physiology of life history trade-offs in
2 animals. *Ann. Rev. Ecol. Syst.* 32:95-126.
- 3 Zera, A. J., and Z. Zhao. 2004. Effect of a juvenile hormone analogue on lipid
4 metabolism in a wing-polymorphic cricket: implications for the endocrine-
5 biochemical bases of life-history trade-offs. *Physiol. Biochem. Zool.* 77:255-66.
- 6 Zwaan, B., R. Bijlsma, and R. F. Hoekstra. 1995. Direct selection on life span in
7 *Drosophila melanogaster*. *Evolution* 49:649-659.
- 8
- 9
- 10
- 11
- 12
- 13
- 14
- 15
- 16
- 17
- 18
- 19
- 20
- 21
- 22

1 TABLE 1. Repeated-measures MANOVA for egg-to-adult viability (proportion egg-to-
 2 adult survival) measured after 7, 14, and 19 generations of selection. Also see FIGURE 1.

3	<hr/>					
4	Source	Roy's greatest root	<i>F</i>	d.f. num	d.f. den.	<i>P</i>
5	<hr/>					
6	Among treatments					
7	JHa treatment	1.03	28.7	1	28	<0.0001
8	Selection regime	0.02	0.59	1	28	0.44
9	JHa × regime	0.26	7.29	1	28	0.012
10	Within treatments					
11	Time	352.8	4762.8	2	27	<0.0001
12	Time × JHa	0.29	3.97	2	27	0.038
13	Time × regime	0.12	1.56	2	27	0.22
14	Time × JHa × regime	0.15	2.00	2	27	0.15
15	<hr/>					
16						
17						

1 TABLE 2. Survival and mortality statistics. Mortality parameters λ (frailty) and γ (rate of aging) were estimated from the
 2 Gompertz model using maximum likelihood estimation (MLE). Parameters were compared among cohorts stratified by JHa
 3 treatment and selection regime (A vs. B; C vs. D; A vs. C; and B vs. D) using log-likelihood ratio tests. Shared superscripts
 4 denote non-significant comparisons; all significant results ($P < 0.001$) remained significant after Bonferroni correction for
 5 multiple comparisons. Starting values for the MLE procedure were $\lambda = 0.0001$ and $\gamma = 0.10$. Analyzing sexes separately did
 6 not change the results; sexes were thus pooled. Cohort size is the initial cohort size (total initial number of flies per treatment,
 7 pooled across replicate cages). Adult life expectancy (days) was estimated from eclosion; median lifespan is the age (in days)
 8 at which half the subjects have failed. Also see FIGURE 2.

9						
10	Cohort	λ	γ	Cohort size	Median lifespan	Life expectancy
11						
12	A) Control, no JHa	0.002	0.076 ^a	366	44	39.5
13	B) Control, JHa	0.013	0.044	388	26	27.3
14	C) Selection, no JHa	0.001 ^a	0.085 ^{a,b}	357	46	43.3
15	D) Selection, JHa	0.002 ^a	0.082 ^b	348	44	40.0

1 TABLE 3. Log-rank tests for differences in adult survival (fraction of flies alive), stratified by JHa treatment and selection
 2 regime. All results remain significant after Bonferroni correction for multiple comparisons. Also see FIGURE 2.

3	<hr/>			
4	5	6	7	8
	Comparison between cohorts	Effect	χ^2	<i>P</i>
	<hr/>			
6	7	8	9	10
	A, B: Control (no JHa), Control (JHa)	Effect of JHa in Control	87.4	<0.0001
	8	9	10	11
	C, D: Selection (no JHa), Selection (JHa)	Effect of JHa in Selection	13.2	0.0003
	9	10	11	12
	A, C: Control (no JHa), Selection (no JHa)	Effect of Selection without JHa	12.4	0.0004
	10	11	12	13
	B, D: Control (JHa), Selection (JHa)	Effect of Selection with JHa	88.8	<0.0001
	<hr/>			

11

12

13

14

15

16

1 TABLE 4. Repeated-measures MANOVA for fecundity (average number of eggs laid per
 2 female per 48-hour interval) over the first 10 days posteclosion. Also see FIGURE 3.

3	<hr/>					
4	Source	Roy's greatest root	<i>F</i>	d.f. num	d.f. den.	<i>P</i>
5	<hr/>					
6	Among treatments					
7	JHa treatment	0.2	5.61	1	28	0.025
8	Selection regime	0.00008	0.002	1	28	0.96
9	JHa × regime	0.0096	0.27	1	28	0.61
10	Within treatments					
11	Time	3.92	25.54	4	25	<0.0001
12	Time × JHa	0.02	0.12	4	25	0.97
13	Time × regime	0.26	1.63	4	25	0.19
14	Time × JHa × regime	0.25	1.54	4	25	0.22
15	<hr/>					
16						

1 TABLE 5. ANOVA for developmental time (hours). Because of the unbalanced nature of
 2 the data, Satterthwaite's approximation was used to construct approximate degrees of
 3 freedom and *F*-tests. Also see FIGURE 4 A.

4	5	6	7	8	9
Source	<i>F</i>	d.f. num	d.f. den.	<i>P</i>	
8	JHa treatment	242.24	1	14.07	<0.0001
9	Selection regime	1.98	1	5.52	0.21
10	JHa × regime	1.71	1	14.07	0.21
11	Replicate line (Regime)	3.60	14	5.50	0.07
12	JHa × Line (Regime)	1.95	14	14	0.11
13	Sex	15.25	1	14.30	0.002
14	Sex × JHa	4.27	1	14.13	0.06
15	Sex × Regime	0.01	1	14.30	0.92
16	Sex × JHa × Regime	0.38	1	14.13	0.55
17	Sex × Line (Regime)	0.45	14	14	0.93
18	Sex × JHa × Line (Regime)	1.00	14	62	0.46

19

1 TABLE 6. ANOVA for body weight at eclosion (mg). Because of the unbalanced nature of
 2 the data, Satterthwaite's approximation was used to construct approximate degrees of
 3 freedom and F -tests. Also see FIGURE 4B.

4	<hr/> <hr/>				
5	Source	F	d.f. num	d.f. den.	P
6	<hr/>				
7	JHa treatment	72.24	1	14	<0.0001
8	Selection regime	0.93	1	14	0.35
9	JHa \times regime	0.28	1	14	0.61
10	Replicate line (Regime)	1.26	14	18.84	0.31
11	JHa \times Line (Regime)	6.29	14	14	<0.001
12	Sex	383.80	1	14	<0.0001
13	Sex \times JHa	106.90	1	14	<0.0001
14	Sex \times Regime	0.22	1	14	0.65
15	Sex \times JHa \times Regime	5.00	1	14	0.04
16	Sex \times Line (Regime)	2.80	14	14	0.03
17	Sex \times JHa \times Line (Regime)	0.31	14	64	0.99
18	<hr/>				

FIGURE CAPTIONS

1

2

3 FIGURE 1. Egg-to-adult viability (proportion surviving) of selected lines and unselected
4 control lines as a function of JHa treatment. Data shown are means \pm standard errors (SE)
5 of replicate lines within a selection regime, averaged across three viability assays
6 performed after 7, 14, and 19 generations of selection. JHa treatment reduced egg-to-
7 adult viability in unselected JHa-susceptible control flies, but not in selected flies which
8 evolved resistance to JHa. Also see TABLE 1.

9

10 FIGURE 2. Adult survivorship and age-specific mortality rates of selected lines and
11 unselected control lines as a function of JHa treatment. (A) Adult survivorship (fraction
12 of flies alive, l_x), for both sexes pooled. JHa treatment strongly reduced survivorship of
13 unselected JHa-susceptible control flies, but only moderately decreased survival of
14 selected JHa-resistant flies. Treatment of JHa-resistant flies with JHa restored longevity
15 to the level seen in unselected control flies not treated with JHa (compare solid triangles
16 with open squares). As compared to unselected control flies, selected flies evolved
17 increased adult survival and extended median lifespan. Together, these data suggest that
18 JHa shortens lifespan. See TABLES 2 and 3 for survival statistics. (B) Age-specific
19 mortality rates (natural logarithm of μ_x), for both sexes pooled. For clarity, mortality rates
20 were smoothed using running averages over three census intervals (6 days). JHa
21 treatment strongly increased frailty (λ) in unselected control flies, but not in selected JHa-
22 resistant flies, suggesting that JH increases the baseline susceptibility of individuals to

1 death. Selected flies evolved decreased frailty across age classes relative to unselected
2 control flies. See TABLE 2 for mortality statistics.

3
4 FIGURE 3. Early fecundity over the first 10 days posteclosion (average number of eggs
5 laid per female per 48 hour interval, \pm SE) of selected lines and unselected control lines
6 as a function of JHa treatment. JHa treatment significantly increased fecundity in
7 unselected control flies, confirming the reproductive function of JH. In contrast, JHa had
8 no effect on the reproductive output of selected JHa-resistant flies. See TABLE 4 for
9 fecundity statistics.

10
11 FIGURE 4. Developmental time (A, in hrs) and body weight at eclosion (B, in mg) of
12 selected and unselected control lines as a function of JHa treatment. Data shown are
13 means \pm standard errors (SE). JHa treatment prolonged developmental time and reduced
14 weight at eclosion, both among selected and unselected control lines. However, the JHa \times
15 selection regime interaction was non-significant for both traits; thus neither trait showed a
16 correlated response to selection. See TABLES 5 and 6 for statistical analyses of
17 developmental time and body weight data.

18

19

20

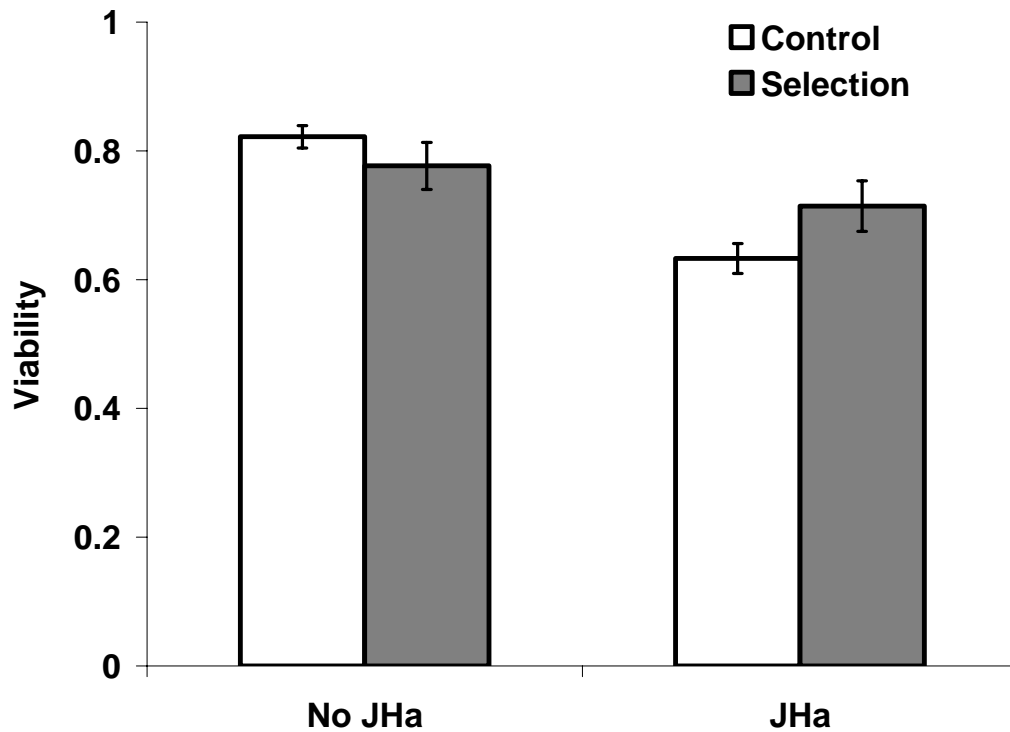
21

22

23

1 FIGURE 1.

2



3

4

5

6

7

8

9

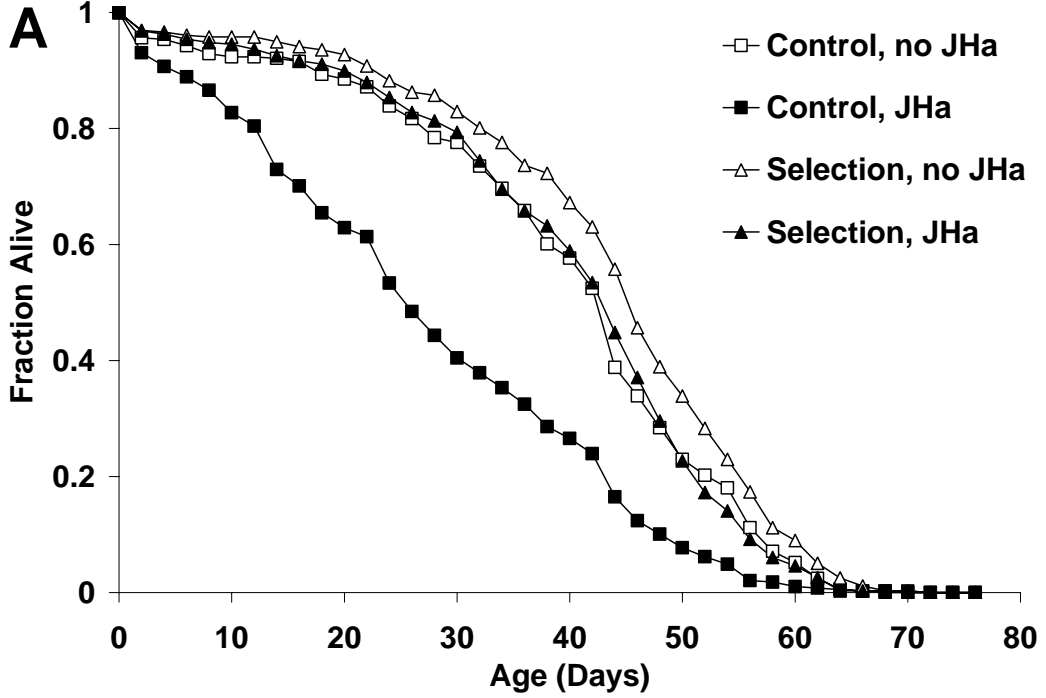
10

11

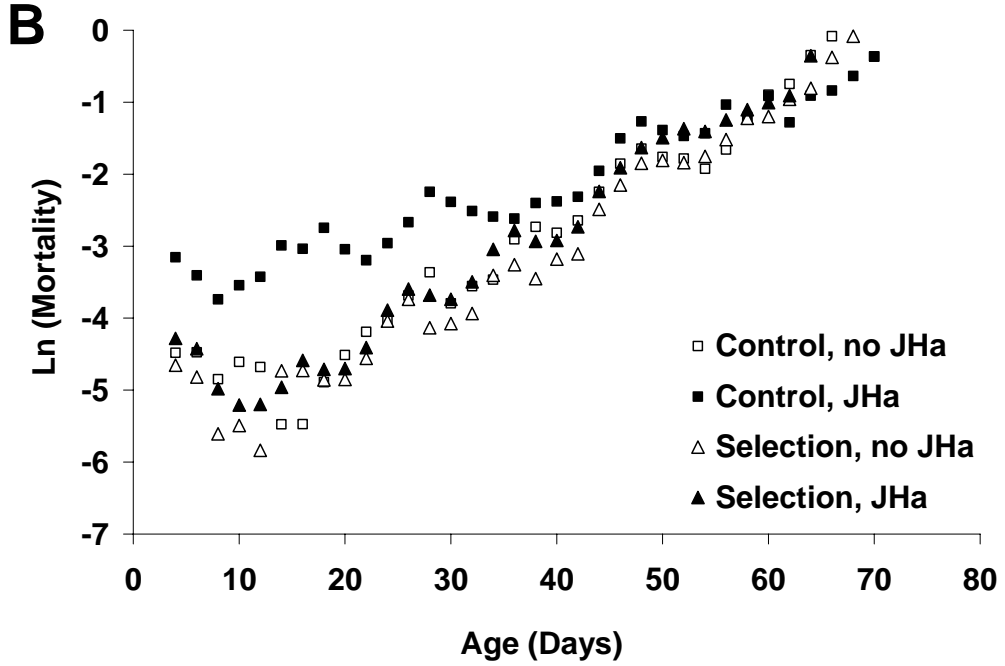
12

1 FIGURE 2.

2

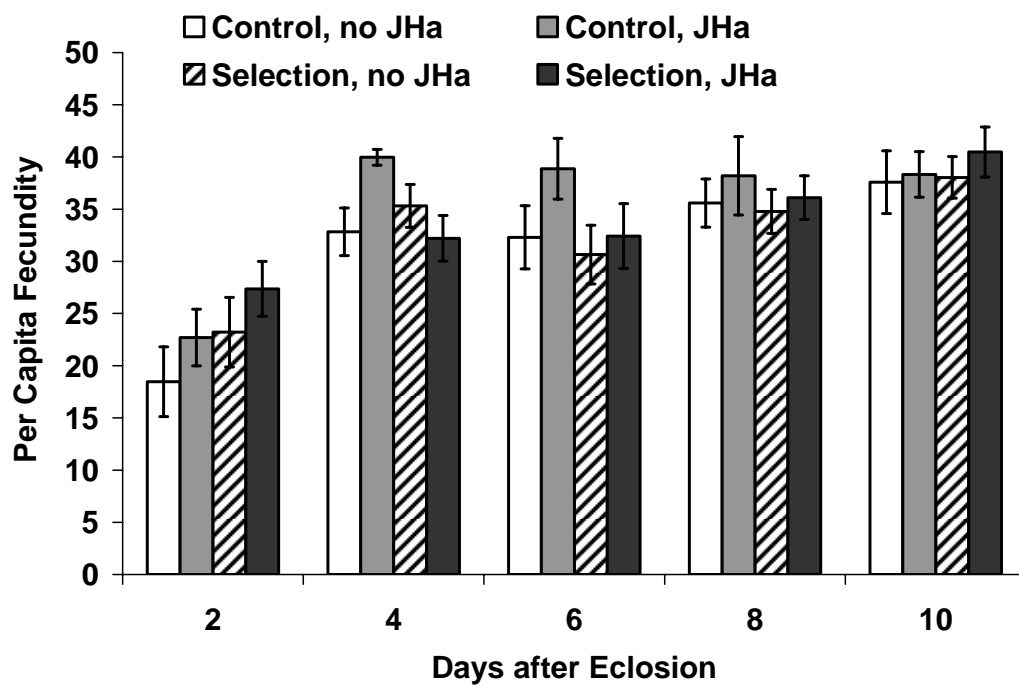


3



4

1 FIGURE 3.



2

3

4

5

6

7

8

9

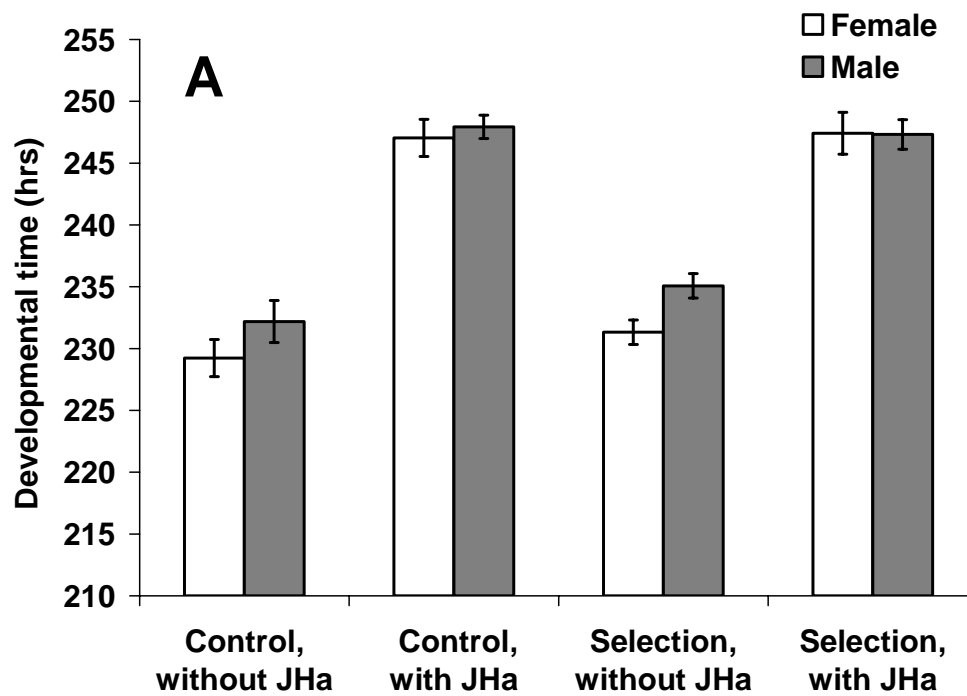
10

11

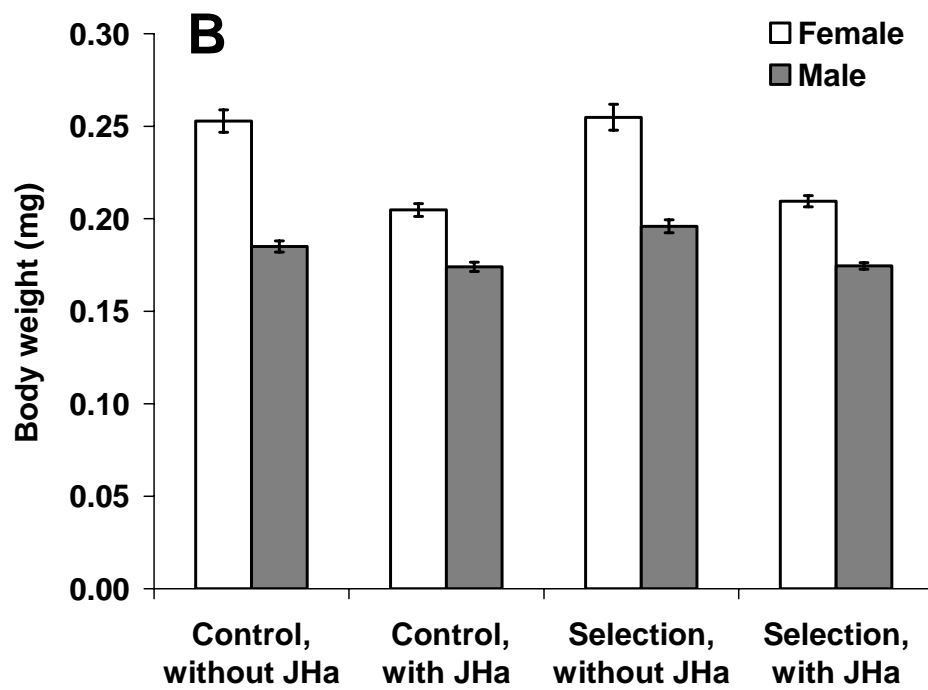
12

13

1 FIGURE 4.



2



3